

In the Claims:

1. (currently amended) A method for differentiating one or more pluripotent embryonic stem (ES) cells comprising:
  - a. culturing the ES cells at low density in a serum-free and feeder-layer free media comprising leukemia inhibitory factor; and
  - b. allowing said ES cells to differentiate to primitive neural stem cells.
2. (previously amended) The method according to claim 1 for differentiating embryonic stem cells to cells with markers characteristic of neural cells comprising:
  - a. culturing the embryonic stem cells in the serum free and feeder-layer free media at low cell density wherein said density is selected to minimize ES cell aggregation or EB formation; and
  - b. allowing said cells to differentiate.
3. (previously amended) The method of claim 2 wherein the cell density is selected as to avoid EB formation.
4. (original) The method of claim 1 wherein said cell density is greater than 0 cells/ $\mu$ l to 50 cells/ $\mu$ l.
5. (original) The method of claim 4 wherein the cell density is greater than 0 cells/ $\mu$ l to 20 cells/ $\mu$ l.
6. (original) The method of claim 5 wherein the cell density is greater than 0 cells/ $\mu$ l to 10 cells/ $\mu$ l.
7. (original) The method of claim 6 wherein the cell density is 10 cells/ $\mu$ l.
8. (original) The method of claims 6 wherein there is no EB formation.
9. (previously amended) The method of claim 7 wherein the differentiating ES cells form at least one sphere colony.
10. (previously amended) The method of claim 1 wherein the differentiating ES cells form at least one sphere colony.
11. (original) The method of claim 1 wherein the serum free media further comprises a cytokine.
12. (canceled)

13. (currently amended) The method of claim 121 wherein the primitive neural stem cells are pluripotent.

14. (currently amended) The method of claims 1 or 12 wherein the serum free media further comprises a growth factor.

15. (original) The method of claim 14 wherein the growth factor is selected from the members of the fibroblast growth factor (FGF) family of growth factors.

16. (original) The method of claim 15 wherein the growth factor is FGF2.

17. (currently amended) The method according to claim 1 wherein the media comprises Noggin or a compound from the Cerberus family of proteins an inhibitor of TGF- $\beta$  superfamily signal transduction.

18. (canceled)

19. (canceled)

20. (currently amended) A method for producing secondary primitive neural stem cell colonies comprising:

- a. culturing ES cells in low cell density serum-free and feeder-layer free media comprising leukemia inhibitory factor for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;
- b. dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
- c. administering a growth factor or survival factor to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

21. (original) A method according to claim 20 wherein the growth factor is selected from among the members of the fibroblast growth factor (FGF) family of growth factors.

22. (original) A method according to claims 21 wherein the growth factor is FGF2.

23. (canceled)

24. (canceled)

25. (previously amended) An isolated primitive neural stem cell expressing one or more neural precursor cell marker and/or one or more neural-specific mRNA molecule, and having multilineage potential.

26. (original) A cell according to claim 25 wherein the neural precursor marker nestin is expressed.

27. (original) A cell according to claim 25 or 26 wherein the neural-specific mRNA molecule is Emx2 or HoxB1.

28. (currently amended) A method according to of claims 1 or 12-for analyzing the role of genes in the regulation of neural fate specification.

29. (currently amended) An isolated primitive neural stem cell produced by the method of claim 121 that comprises neural cell markers and is pluripotent.

30. (previously amended) An isolated primitive neural stem cell.

31. (currently amended) A method of producing a pre-selected cell type derived from a cell of claim 30 comprising, culturing the cells in media comprising leukemia inhibitory factor under differentiating conditions that promote formation of the cell type.

32. (original) The method of claim 31 wherein the pre-selected cell type is a neural cell, and the differentiating conditions comprise culturing the cell in a serum free media that comprises FGF2.

33. (currently amended) A method for screening for modulators of primitive neural stem cell differentiation comprising:

- a. culturing primitive neural stem cells in serum-free and feeder-layer free media comprising leukemia inhibitory factor under low density conditions in the presence of the potential modulator; under conditions that produce differentiation in the absence of the modulator;
- b. detecting any differentiation of the cells and cell types generated, if any;
- c. determining whether the modulator affects the differentiation of the cells.

34. (original) A method in accordance with claim 33, wherein the modulators comprise any culturing conditions that may modulate cellular differentiation.

35. (currently amended) A method for screening for differentiation factors of cellular development comprising:

- a. culturing the cells in serum free media comprising leukemia inhibitory factor at low cell density in the presence of the differentiation factor;
- b. allowing cells to differentiate;
- c. detecting differentiation of the cells, if any.

36. (previously amended) A method of claim 35 for screening for differentiation factors of neural cell development.

37. (currently amended) A method for screening for differentiation factors of cellular development comprising:

- a. culturing the cells of claim 29 in serum free media comprising leukemia inhibitory factor, in the presence of the differentiation factor.
- b. detecting any differentiation of the cells.

38. (original) The method of claim 37, wherein the media further comprises FGF2.

39. (canceled)

40. (previously amended) A method according to claim 38 for modulating cellular differentiation.

41. (previously amended) The method of claim 1 for obtaining a homogenous uniform cell base.

42. (previously amended) The method of claim 29 wherein the cell base is a neural cell base.

43. (currently amended) A method for supplying cells for transplantation comprising culturing cells pursuant to the method of claim 1-~~or 12~~.

44. (canceled)

45. (canceled)

46. (canceled)

47. (currently amended) A method for producing secondary primitive neural stem cell colonies comprising:

- a. culturing ES cells in low cell density serum-free and feeder-layer free media comprising leukemia inhibitory factor for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;
- b. dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
- c. administering LIF or B27 to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

48. (previously added) The primitive neural stem cell of claim 30, wherein the cell is isolated from an embryonic stem cell.

49. (previously added) An isolated sphere colony comprising primitive neural stem cells.

50. (canceled)